PCT / SE 2004 / 0 0 1 3 6 9

2 4 -09- 2004

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

Signed

Dated 2 August 2004

Patents Form 1/77

Pat[illegible] Act 1977
[illegible]le 16)

The Patent Office

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

26 SEP 2003

The Patent Office
Cardiff Road
Newport
South Wales
NP10 8QQ

THE PATENT OFFICE
26 SEP 2003
RECEIVED BY HAND

1. Your reference — 80177879/001

2. Patent application number *(The Patent Office will fill in this part)* — 0322645.3

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Melacure Therapeutics AB
Ulleråkersvägen 38
SE-756 43 Uppsala
Sweden

295EP03 E840394-17 D00027
P01/7700 0.00-0322645.3

Patents ADP number *(if you know it)* 08566010001

If the applicant is a corporate body, give country/state of its incorporation: SE

4. Title of the invention: Use of antisecretory factor peptides

5. Name of your agent *(if you have one)*: Frank B. Dehn & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*:
179 Queen Victoria Street
London
EC4V 4EL

Patents ADP number *(if you know it)*: 166001

6. Priority: Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. Divisionals, etc: Complete this section only if this application is a divisional application or resulted from an entitlement dispute *(see note f)*

Number of earlier UK application	Date of filing *(day / month / year)*

8. Is a Patents From 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request?

Answer YES if:
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
Otherwise answer NO (See note d)

Yes

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form	0
Description	18
Claim(s)	8
Abstract	1
Drawing(s)	6 + 6

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for a preliminary examination and search *(Patents Form 9/77)*	1
Request for a substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	1 Page of Sequence Listing

11. I/We request the grant of a patent on the basis of this application.

Signature Philip Webber Date 26 September 2003

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

Philip Webber
01273 244200

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*
b) *Write your answers in capital letters using black ink or you may type them.*
c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s) of the form. Any continuation sheet should be attached to this form.*
d) *If you have answered 'Yes', Patents Form 7/77 will need to be filed.*
e) *Once you have filled in the form you must remember to sign and date it.*
f) *Part 7 should only be completed when a divisional application is being made under section 15(4), or when an application is being made under section 8(3), 12(6) or 37(4) following an entitlement dispute. By completing part 7, you are requesting that this application takes the same filing date as an earlier UK application. If you want the new application to have the same priority date(s) as the earlier UK application, you should also complete part 6 with the priority details.*

77879UK1

USE OF ANTI-SECRETORY FACTOR PEPTIDES

The present invention relates to the use of polypeptides comprising certain fragments of anti-secretory factor (AF) in the manufacture of a medicament for treating and/or preventing a condition that is characterised by or associated with the pathological loss and/or gain of cells, such as the pathological degeneration, loss of regeneration ability and/or loss of control of regeneration of a differentiated cell and/or tissue, an embryonic stem cell, an adult stem cell, a progenitor cell and/or a cell derived from a stem cell or progenitor cell. Medical conditions characterised by or associated with such conditions include damage to the brain by trauma, asphyxia, toxins, hypoxia, ischemia, infections or degenerative or metabolic insults resulting in neurological and/or cognitive defects.

Traumatic, asphyxial, hypoxic, ischemic, toxic, infectious, degenerative or metabolic insults to the central nervous system (CNS) often result in damage to several different cell types. As an example of a degenerative insult to the CNS, Parkinson's disease often causes loss of specific populations of cells and is in particular associated with the specific loss of dopaminergic neurons in the Substantia nigra. Similarly, multiple sclerosis is associated with loss of myelin and oligodendrocytes. Another illustration of a degenerative disorder caused by selective loss of a specialised type of neurons is Alzheimer's disease, which is associated with loss of cholinergic neurons. Additionally, there are many other instances in which CNS injuries or diseases can cause damage to oligodendroglia, astroglia, or neuronal cells.

In general, replacement of neurons following degeneration or damage is not a characteristic of the adult mammalian brain. Neuronal loss is thus usually considered permanent. Nonetheless, prolonged postnatal neurogenesis has been described in the granule cell layer of the hippocampal formation to persist well into adulthood in several species, including man (Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A., Nordborg, C., Peterson, D.A., and Gage, F.H., Nature Med. 11: 1313-1317 (1998)). This phenomenon is attributed to the existence of an omnipotent neuronal cell population, the neuronal progenitor cells. Neuronal progenitor cells are stem cells and reside in the subgranular zone (SGZ) of the dentate gyrus where they continuously proliferate, migrate into the granula cell layer and differentiate into granule cells. The

new-born neurons in the granule cell layer express markers of differentiated neurons and have morphological characteristics corresponding to differentiated granula cells. Furthermore, they establish axonal processes into the mossy fibre pathway and form synaptic connections with their targets in hippocampal layer CA3 (Gage, F.H. Science 287:1433-1438 (2000)). The neurogenesis in the dentate gyrus is in itself especially intriguing as the hippocampal formation in the dentate gyrus is intimately associated with spatial learning and memory (McNamara, R.K. and Skelton, R.W., Brain Res. Rev. 18: 33-49 (1993)). It has previously been shown that the proliferation of progenitor cells in the SGZ can be influenced by the administration of n-methyl-d-aspartate (NMDA) receptor antagonists or by the removal of the adrenal glands (Cameron, H.A. and Gould, E., Neuroscience 61: 203-209 (1994); Cameron, H.A., Tanapat, P. and Gould, E., Neuroscience 82: 349-354 (1998)). What is more, it has been shown that exposure to enriched environments leads to an increased number of surviving, newly formed granula cells as well as to an increased total number of neurons in the dentate gyrus (Kempermann, G., Kuhn, H.G. and Gage, F.H., Nature 386: 493-495 (1997)).

In addition to the potential of replacing neurons following degeneration or damage, neuronal progenitor cells are also thought to be important for the ability to learn. In accordance with the fact that neuronal plasticity is reduced with increased age, recent studies have demonstrated that proliferation of progenitor cells is also decreased (Kuhn, H., Dickinson-Anson, H., and Gage, F.H., J. Neurosci. 16: 2027-2033 (1996)).

Interestingly, stem cells that were isolated from the adult rodent brain have been transplanted into the hippocampus of adult animals where they differentiated into cells with neuronal characteristics (Suhonen, J.O., Peterson, D.A., Ray, J., and Gage, F.H., Nature 383:624-627 (1996)). Moreover, stem cells from one germinal layer were in addition seen to be able to form tissue from a different germinal layer (Anderson DJ, Gage FH, Weissman I.L. Nat Med. 4:393-395 (2001)).

Stem cells have successfully been isolated from different tissue sources (Gage F.H. Science. 287:1433-1438 (2000)) and even human embryonic stem cell lines (ES-cells) have been established from pre-implantation embryos (Thomson et al., Science 282: 1145-1147 (1998). These ES-cells were shown to maintain a stable developmental potential to form advanced derivatives of all three embryonic germ layers. As is easily understood, human ES cell lines and stem cell lines derived from different tissues like

skin, blood or brain have widespread applications for human developmental biology, drug discovery, drug testing and transplantation medicine.

Unfortunately, in order to facilitate autologous transplantation of neuronal cells, it is still necessary to use adult-derived cells which are complex to isolate and to propagate.

Anti-secretory factor (AF) is a polypeptide which occurs naturally in human body fluids (Bjorck, S., Bosaeus, I., Ek, E., Jennische, E., Lonnroth, I., Johansson, E., and Lange S., Gut 46(6):824-829 (2000)). AF known to inhibit intestinal fluid secretion induced by cholera toxin (Johansson, E., Lonnroth, I., Lange, S., Jonson, I., Jennische, E., and Lonnroth, C., J. Biol. Chem. 270(35):20615-20620 (1995)) and it has also been shown to suppress intestinal inflammation and hypersecretion in rat induced by toxin A from Clostridium difficile (Johansson, E., Jennische, E., Lange, S., and Lonnroth, I., Gut 41(5) 719-720 (1997)). AF is a 41 kDa protein consisting of 382 amino acids. It appears as a 60 kDa protein on SDS-polyacrylamide gels (Johansson, E., Lonnroth, I., Lange, S., Jonson, I., Jennische, E., and Lonnroth, C., J. Biol. Chem. 270(35):20615-20620 (1995)). The active site has been located in a small region of the AF-sequence, between amino acids 36 and 42 (Johansson, E., Lange, S., and Lonnroth, I., Biochim. Biophys. Acta 1362 (2-3):177-182) 1997). Anti-secretory factor peptides regulating pathological permeability changes are described in WO 97/08202. However, no effect on proliferation or differentiation in any cell type is disclosed in the aforementioned publication.

The present inventors have now surprisingly found that certain fragments of AF are capable of inducing proliferation of progenitor cells derived from the adult CNS. This suggests a new and exiting mode of action involving AF fragments in mediating the survival, proliferation and/or propagation of these cells in vitro as well as in vivo. In particular, the inventors have found that certain AF fragments can modulate the proliferation, differentiation, migration and/or programmed cell death of a neural stem cell or progenitor cell from the adult CNS. The present invention thus provides new and improved means to treat injuries to or diseases or disorders of the central or peripheral nervous system, inter alia, and provides possibilities for influencing the neuronal plasticity of the CNS.

The mammalian brain, including the human brain, retains its ability to generate neurons throughout life in certain regions only. New neurons and astroglial cells and oligodendrocytes are generated by cell genesis from stem or progenitor cells. During

the research leading to the present invention, it was found that certain AF fragments induce an increase in cell genesis from progenitors and/or stem cells *in vitro* as well as in the adult brain.

It has now been found that it is possible to treat neural loss suffered after a CNS insult or in the progress of a neuronal disease or disorder, by increasing the number of stem cell or progenitor derived cells, including neurons, astroglial cells and/or oligodendrocytes, by administering an effective amount of certain AF fragments. The concentration of the AF fragment may be increased in the CNS of the patient with the intention of inducing proliferation and/or differentiation of stem cells or progenitor cells with a concomitant increase in cell genesis. It is thus possible to effect cell genesis from stem cells or progenitor cells and thus induce the genesis of neurons and/or glial cells after either neuronal, oligodendroglial or glial cell loss in the CNS or PNS, or to prevent the normal age-related deterioration of said cells in the CNS or PNS.

One embodiment of the invention relates to the use of a polypeptide comprising an amino acid sequence of Formula I:

X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent
X2 is H, R or K
X3 is S or L
X4 is T or A
X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent,
or a pharmaceutically acceptable salt thereof,

in the manufacture of a medicament for the treatment and/or prevention of a condition associated with or characterised by a pathological loss and/or gain of cells.

Preferably, Formula I has the sequence of one of:

a) amino acids nos. 35-42 of SEQ ID NO:1
b) amino acids nos. 35-46 of SEQ ID NO:1
c) amino acids nos. 36-51 of SEQ ID NO:1
d) amino acids nos. 36-80 of SEQ ID NO:1
e) amino acids nos. 1-80 of SEQ ID NO:1,

or a pharmaceutically acceptable salt thereof.

SEQ ID NO:1 is the first part of the amino acid sequence of the anti-secretory factor polypeptide as given in Johansson, E., et al., Molecular cloning and expression of a pituitary gland protein modulating intestinal fluid secretion. J Biol. Chem., 1995. 270(35): p. 20615-20; WO9708202 and US 6,344,440.

The invention also relates to uses of pharmaceutically acceptable salts of the polypeptides comprising an amino acid sequence of Formula I.

The invention also provides a method of treating and/or preventing a condition associated with or characterised by a pathological loss and/or gain of cells, comprising the administration to a patient of an effective amount of a polypeptide comprising an amino acid sequence of Formula I.

The invention also provides a polypeptide comprising the amino acid sequence of Formula I for use in treating and/or preventing a condition associated with or characterised by a pathological loss and/or gain of cells.

In some embodiments of the invention, the polypeptides of Formula I may additionally comprise terminal protecting groups. Examples of N-terminal protecting groups include acetyl. Examples of C-terminal protecting groups include amide.

The term "pathological loss and/or gain of cells" is in the present context used to describe the common technical feature of a number of medical conditions and disorders. These conditions and disorders are characterised by displaying pathological degeneration of, loss of ability of regeneration of and/or loss of control of regeneration of a differentiated cell and/or tissue, an embryonic stem cell, an adult stem cell, a progenitor cell and/or a cell derived from a stem cell or progenitor cell.

The condition to be treated may be caused, inter alia, by one or more of traumatic, asphyxia, neuropathic pain, hypoxic, ischemic, toxic, infectious, degenerative or metabolic insults to the central nervous system (CNS). These often result in damage to several different cell types. Thus, damage to the brain by trauma, asphyxia, toxins, ischemia or infections frequently causes neurological and/or cognitive defects. In other cases, the condition may be caused by a traumatic, malignant, inflammatory, auto-immune or degenerative disorder. In yet other cases, the condition may be caused by

genetic factors or the cause may be unknown. In yet further cases, the condition may be caused by axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS, and/or damage to the spinal cord after disease and/or trauma.

In one embodiment of the invention, the condition to be treated is a condition that is associated with or characterised by a pathological loss and/or gain of cells in the peripheral nervous system (PNS) or central nervous system (CNS).

Cells that may be affected by a polypeptide comprising an amino acid sequence of Formula I are, for example, embryonic stem cells, adult stem cells, progenitor cells and/or cells derived from them. They can belong to any or all of the three germinal layers. Once stimulated, the above-listed stem cells will differentiate into and/or replace malfunctioning, dying, or lost cells or cell populations, such as in pathological CNS or PNS conditions characterised by abnormal loss of cells, oligodendroglia, astroglia, and/or neuronal cell and/or cell population, such as non-cholinergic neuronal cell, cholinergic neuronal cell, and/or glial cell and/or cell populations.

The invention particularly relates therefore to the treatment of conditions associated with or characterised by a loss and/or gain of stem cells, preferably neural stem cells, for example adult or embryonic stem cells; or conditions associated with or characterised by a loss and/or gain of progenitor cells. In one embodiment, the stem cells are hippocampal stem cells, preferably adult-derived hippocampal stem cells.

The invention also relates particularly to the treatment of conditions associated with a loss and/or gain of differentiated cells. In one preferred embodiment, the differentiated cells are chondrocytes, cardiomyocytes, oligodendroglia, astroglia, neuronal cells, epithelial cells, endothelium, skin, blood, liver, kidney, bone, connective tissue, lung tissue, exocrine gland tissue and/or endocrine gland tissue or muscle cells. Preferably, the differentiated cells are neural cells, neurons, astrocytes, oligodendrocytes, Schwan cells or glial cells. The neural cells may, inter alia, be non-cholinergic neuronal cells or cholinergic neuronal cells.

The invention also provides the use of a polypeptide comprising an amino acid sequence of Formula I in the manufacture of a medicament for modulating the development of stem cells and/or progenitor cells.

A further embodiment of the invention provides a method of modulating the development of stem cells and/or progenitor cells, comprising contacting the stem cells and/or progenitor cells in vivo or ex vivo with an amount of a polypeptide comprising an amino acid sequence of Formula I.

The invention further provides a polypeptide comprising an amino acid sequence of Formula I for use in modulating the development of stem cells and/or progenitor cells in vivo or ex vivo.

In the context of the present invention, the term "modulating the development of stem cells and/or progenitor cells" includes one or more of inducing proliferation, inducing programmed cell death, maintaining the genesis, promoting differentiation and promoting migration of stem cells and/or progenitor cells.

The uses and methods of the invention are preferably suited for the treatment of abnormal and/or medical conditions affecting pathological loss or gain of neural stem cells, progenitor cells and/or cells derived from neural stem cells or progenitor cells. They may thus be used to prevent, treat or ameliorate damage, diseases or deficits of the CNS or the PNS. The pharmaceutically active substance used according to the invention is especially suitable for treatment of conditions affecting the oligodendroglia, astroglia, and/or neuronal cells. Such conditions may, for example, be due to CNS damage or deficits, neuronal cell loss or memory loss. Such conditions may be caused by a number of different factors or diseases, e.g. traumatic, malignant, inflammatory, auto-immune or degenerative disorders such as multiple sclerosis, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, Meniere's disease and demyelition disorder. The effect of the pharmaceutically active substances used according to this preferred embodiment of the invention is due to their ability either to induce cell genesis, proliferation and/or differentiation of progenitor derived cells in or from the central nervous system.

Nonetheless, and as outlined above, the present invention is not restricted to uses and methods for treating neuronal diseases and conditions, but said uses and methods may also be used for treating a large variety of mammalian conditions that are characterised by pathological cell loss and/or gain, such as Parkinson's disease (dopaminergic neurons), Alzheimer's disease (cholinergic neurons), Multiple Sclerosis (oligodendrocytes), stroke (neurons and glial cells), asphyxia or hypoxia (neurons and glial cells), heart failure (cardiomyocytes), heart infarction (cardiomyocytes), diabetes

(pancreatic beta cells), artrosis or arthritis (chondrocytes), skin disease and burn injuries (dermis and epidermis), liver diseases or failure (hepatocytes), muscle diseases or damages (myocytes), cancer (tissues affected by cancer), pancreatic dysfunction (exocrine or endocrine pancreatic cells) and inflammatory bowel disease (intestinal cells).

The invention further relates to a pharmaceutical composition comprising a polypeptide comprising the amino acid sequence of Formula I for use in treating a condition associated with or characterised by a pathological loss and/or gain of cells. Additionally, the invention provides a pharmaceutical composition comprising a polypeptide comprising an amino acid sequence of Formula I for use in modulating the development of stem cells and/or progenitor cells in vivo or ex vivo.

The pharmaceutical compositions or medicaments of the invention may additionally comprise one or more pharmacologically acceptable carriers, excipients or diluents, such as those known in the art.

The compositions or medicaments may be in form of, for example, fluid, semi-solid or solid compositions such as, for example but not limited to dissolved transfusion liquids, such as sterile saline, Ringer's solution, glucose solutions, phosphate buffered saline, blood, plasma or water, powders, microcapsules, microspheres, nanoparticles, sprays, aerosols, inhalation devices, solutions, dispersions, suspensions, emulsions and mixtures thereof.

The compositions or medicaments may be formulated according to conventional pharmaceutical practice, see, e.g., "Remington: The science and practice of pharmacy" 20th ed. Mack Publishing, Easton PA, 2000 ISBN 0-912734-04-3 and "Encyclopedia of Pharmaceutical Technology", edited by Swarbrick, J. & J. C. Boylan, Marcel Dekker, Inc., New York, 1988 ISBN 0-8247-2800-9.

The choice of pharmaceutically acceptable excipients in a composition or medicament for use according to the invention and the optimum concentration thereof may readily be determined by experimentation. Also whether a pharmaceutically acceptable excipient is suitable for use in a pharmaceutical composition is generally dependent on which kind of dosage form is chosen. However, a person skilled in the art of pharmaceutical formulation can find guidance in e.g., "Remington: The science and

practice of pharmacy" 20th ed. Mack Publishing, Easton PA, 2000 ISBN 0-912734-04-3.

A pharmaceutically acceptable excipient is a substance, which is substantially harmless to the individual to which the composition will be administered. Such an excipient normally fulfils the requirements given by the national drug agencies. Official pharmacopoeias such as the United States of America Pharmacopoeia and the European Pharmacopoeia set standards for well-known pharmaceutically acceptable excipients.

The following is a review of relevant pharmaceutical compositions for use according to the invention. The review is based on the particular route of administration. However, it is appreciated that in those cases where a pharmaceutically acceptable excipient may be employed in different dosage forms or compositions, the application of a particular pharmaceutically acceptable excipient is not limited to a particular dosage form or of a particular function of the excipient.

Parenteral compositions:

For systemic application, the compositions according to the invention may contain conventional non-toxic pharmaceutically acceptable carriers and excipients, including microspheres and liposomes.

The compositions for use according to the invention may include all kinds of solid, semi-solid and fluid compositions. Compositions of particular relevance are e.g. solutions, suspensions and emulsions.

The pharmaceutically acceptable excipients may include solvents, buffering agents, preservatives, chelating agents, antioxidants, stabilisers, emulsifying agents, suspending agents and/or diluents. Examples of the different agents are given below.

Examples of various agents:

Examples of solvents include but are not limited to water, alcohols, blood, plasma, spinal fluid, ascites fluid and lymph fluid.

Examples of buffering agents include but are not limited to citric acid, acetic acid, tartaric acid, lactic acid, hydrogenphosphoric acid, bicarbonates, phosphates, diethylamine, etc..

Examples of chelating agents include but are not limited to sodium EDTA and citric acid.

Examples of antioxidants include but are not limited to butylated hydroxy anisole (BHA), ascorbic acid and derivatives thereof, tocopherol and derivatives thereof, cysteine, and mixtures thereof.

Examples of diluents and disintegrating agents include but are not limited to lactose, saccharose, emdex, calcium phosphates, calcium carbonate, calcium sulphate, mannitol, starches and microcrystaline cellulose.

Examples of binding agents include but are not limited to saccharose, sorbitol, gum acacia, sodium alginate, gelatine, starches, cellulose, sodium carboxymethylcellulose, methylcellulose, hydroxypropylcellulose, polyvinylpyrrolidone and polyetyleneglycol.

The pharmaceutical composition or the substance used according to the invention is preferably administered via intravenous peripheral infusion or via intramuscular or subcutaneous injection into the patient or via buccal, pulmonary, nasal or oral routes. Furthermore, it is also possible to administer the pharmaceutical composition or the pharmaceutically active substance through a surgically inserted shunt into a cerebral ventricle of the patient.

In one embodiment of the present invention, said pharmaceutical composition is formulated so that the active substance will pass into the ventricles of a patient's brain or into the cerebrospinal fluid of said patient, when it is administered to said patient. This may e.g. be achieved by mechanical devices, vectors, liposomes, lipospheres, or biological or synthetical carriers.

Preferably, the administered dosage range is about 0.001-100 mg of a polypeptide comprising the amino acid sequence of Formula I per 100 g body weight, comprising a range of 0.001-100 mg/1g, 0.001-100 mg/10g and 0.001-100 mg/50g body weight. Preferably, the administered dosage range is about 0.001-100 mg of a polypeptide comprising the amino acid sequence of Formula I per 1kg body weight.

The invention may be used to treat humans or non-human mammals.

The terms "treatment" or "treating" as used herein relate to both therapeutic treatment in order to cure or alleviate a disease or a medical condition, characterised by abnormal loss and/or gain of cells, and to prophylactic treatment in order to prevent the development of a disease or a medical condition, characterised by pathological loss and/or gain of cells. Thus both prophylactic and therapeutic treatments are included in the scope of the present invention. The terms "treatment" or "treating" also refer to the effecting of cell genesis from stem cells or progenitor cells by inducing the genesis of differentiated cells, such as e.g. neurons and/or glial cells after either neuronal, oligodendroglial or glial cell loss in the CNS or PNS, or to prevent the normal age-related deterioration in the CNS or PNS or other organs of the body. The treatment may either be performed in an acute or in a chronic way.

A polypeptide comprising the amino acid sequence of Formula I may also be used for the cultivation of stem cells or progenitor cells. The stem cells or progenitor cells may be used for the concomitant transplantation to a patient in need thereof, e.g. to the CNS, PNS, or other organs of the body, in said patient.

Thus the invention provides a method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny from any germinal layer from a patient, characterised by:

a) administering an effective amount of a polypeptide comprising the amino acid sequence of Formula I to said patient prior to isolating said cell;
b) propagating said isolated cell *in vitro*; followed by
c) transplanting said propagated cells into the same or another patient in need thereof.

The invention also provides a method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny from any germinal layer from a patient, characterised by:

a) isolating said cell and/or stem cell progeny from the patient;

b) administering an effective amount of a polypeptide comprising the amino acid sequence of Formula I to said isolated cell *in vitro* and propagating said cells; followed by

c) transplanting said propagated cells back into the same or another patient in need thereof.

The isolated cells of the above methods may be derived, inter alia, from the CNS and/or PNS of the patient.

AF-expanded stem and/or progenitor cells may be propagated and either pre-differentiated prior to grafting or allowed to differentiate as a result of interactions between the transplanted cells and the host. AF-expanded stem cells and/or progenitor cells may either be administered and/or grafted at a single time, or delivered repeatedly over a prolonged period. This will be especially useful if stem cells and/or progenitor cells are administered to enter the target organ via the bloodstream.

According to another preferred embodiment of the invention, it is possible to use a polypeptide comprising the amino acid sequence of Formula I to propagate progenitor cells or stem cells or other cells in a tissue culture or a cell culture. Such cells may thereafter be used for cell transplantation into a patient suffering from e.g. neuronal cell loss or a condition due to lack of endogenous cells of another type. The cells used to start the culture may either originate from the patient or from another human or animal donor, and may be used in the treatment of a broad variety of diseases and disorders comprising heart diseases such as infarct, diabetes, or in an assortment of neurological diseases and disorders, such as those referred to above.

Thus the invention also provides a method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny in vitro, characterised by treating the isolated cell with a polypeptide comprising the amino acid sequence of Formula I. Preferably, the isolated cell is selected from the group comprising chondrocytes, myocardial cells, blood cells, neurons, oligodendrocytes, astroglial cells, progenitor cells, stem cells and/or cells derived from said cells. In general, the isolated cell will be treated under appropriate conditions and for a time which is sufficient to achieve the desired propagation, induction, reduction and/or maintenance.

When cells are to be removed from a patient for in vitro propagation, it may be advantageous first to increase the number of progenitor cells in the patient. This will better facilitate the subsequent isolation of said cells from patients. The number of progenitor cells are increased by use of the method or pharmaceutical composition according to the invention.

A polypeptide comprising the amino acid sequence of Formula I, may be used alone or conjunction with other medicaments or growth factors such as epidermal growth factor (EGF), fibroblast growth factor 2 (FGF2) or IGF-I, designed to induce cell genesis or proliferation e.g. in the CNS or PNS. A polypeptide comprising the amino acid sequence of Formula I, alone or in conjunction with other medicaments, peptides, growth-factors, steroids, lipids, glycosylated proteins or peptides, used either simultaneously or in sequence, may be used in order to facilitate cell genesis or the generation of specific cell types in vivo or in vitro. It may also be used to induce immature or multipotent cells to activate specific developmental programs as well as specific genes in the aforementioned cells.

By the above mentioned term "cell genesis" is meant the generation of new cells such as neurons, oligodendrocytes, schwan cells and astroglial cells from multipotent cells, progenitor cells or stem cells within the adult CNS or PNS or other organs of the body, in situ or in isolation.

Furthermore, the invention also relates to the therapeutic use of substances that decrease the amount of active AF or naturally occurring analogous of AF in the patient and thus decrease the genesis of new cells, e.g. of oligodendrocytes in patients with axonal or spinal cord injury, such as axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS, and/or damage to the spinal cord after disease and/or trauma. Examples of such substances are drugs, antibodies, compounds, peptides and/or inhibitor of endogenous AF release.

Since AF supports the genesis of new cells and especially neurons in the hippocampus, a structure intimately coupled to learning and memory, a polypeptide comprising the amino acid sequence of Formula I may be used in order to facilitate learning and memory by the genesis of said cells.

Whilst the present invention relates primarily to a method for treating abnormal conditions in the CNS or PNS that are characterised by pathological loss and/or gain of cells, by affecting neural stem cells or progenitor cells, the uses and methods of the invention may be equally useful for treating and/or preventing medical conditions in other organs of the body, provided that said medical conditions characterised by pathological loss and/or gain of cells.

Whilst reference has primarily been made herein to the use of polypeptides comprising an amino acid sequence of Formula I, the invention relates, mutatis mutandis, also to polypeptides consisting essentially of an amino acid sequence of Formula I, and to polypeptides consisting of an amino acid sequence of Formula I.

The polypeptides comprising an amino acid sequence of Formula I may be produced by standard means, including recombinant and synthetic routes.

The invention will be more fully understood when reading the following Examples which are intended merely to illustrate, but not to limit, the scope of the invention.

FIGURE LEGENDS

Figure 1

To study the effect of AF-peptide on proliferation in adult derived hippocampal stem cells both the proportion of cells in mitosis (A) and the total number of cells (B) were counted. Cells in mitosis were positively stained by an antiserum recognising phosphorylated histone H3 while total number of cells were counted using the nuclear dye staining Hoechst 33258. (*** $P < 0.001$, ** $P < 0.01$, $P < 0.05$).

Figure 2

Illustrates proliferation measured as DNA content in adult derived hippocampal stem cell cultures after incubation with different concentrations of AF compared with untreated control cultures (red line) and cultures treated with 20ng/ml of bFGF (well known inducer of proliferation in stem cells). (* $P < 0.05$).

Figure 3

Complete nucleotide sequence of AF taken from Johansson, E., et al., Molecular cloning and expression of a pituitary gland protein modulating intestinal fluid secretion.

J Biol. Chem., 1995. 270(35): p. 20615-20, WO9708202, US6344440. Only the first part of the corresponding amino acid sequence is given.

EXAMPLES

Example 1: Proliferation experiments

For proliferation experiments, adult derived progenitor cells were passaged into polyornithine/laminin coated glass chamber slides (Nunc) at a density of approximately 0.7 x 104 cells per cm2. The cells were incubated for 48 hours in Dulbecco's Modified Eagles Medium / Hams's F12 (DMEM / F12, 1:1) containing N2 supplement (Life Sciences, Täby, Sweden) and 20 ng/ml recombinant human FGF-2 (Pepro Tech, IHC). The medium was then replaced with N2 medium without FGF-2 for another 48 hours. Thereafter the medium was changed to N2 medium where a polypeptide consisting of amino acids 36-51 of SEQ ID NO:1 was added at different concentrations (10, 1, 0.1 and 0.01 ng/ml, respectively) for 48 hours. The cells were washed once in PBS and fixed in 4%-paraformaldehyde for 10 minutes in +4°C and rinsed twice (10 min) with PBS. Cells were incubated with an anti-phospho-histone H3 antisera (1:400, Upstate Biotechnology, Lake Placid, NY). As secondary antisera we used FITC conjugated sheep anti-rabbit (1:200, Boehringer Mannheim). The nuclear dye bisBenzimide 1:100 from stock at 5 µg/ml (Hoechst 33258, Sigma Chemical Co.) were used. FGF-2 was added in parallel with AF for comparison with a known substance that induce proliferation. Cells were counted using a Nikon Microphot-FX fluorescens microscope using a 40 times (40x) ocular. Ten area units (a.u.) were chosen by moving the focus from one end to the other of the coverslip. Cells in one circle using 40x ocular were counted as one a.u. Results are shown in Fig.1. Doubled-blinded counting were performed in Fig.1. The values in the figure are expressed as the mean ± SEM. Statistical analysis between groups were done using factorial analyses of variance followed by a Fisher's post-hoc test.

Example 2: Quantitation of DNA content

The quantification of DNA content was performed according to manufacturer's instruction (Molecular Probes, OR) using the Cyquant DNA assay. Adult derived hippocampal stem cells were cultured at 0.2 x 104 cells/cm2 in 24-hole plates coated with polyornithine and thereafter laminin. The cells were cultured in N2 medium plus

bFGF for two days followed by N2 without bFGF for two days. Thereafter the cells were incubated in N2 medium without bFGF with a polypeptide consisting of amino acids 36-51 of SEQ ID NO;1 for 48 h, washed once in PBS and stored in -80 C freezer. Cells were thawed and resuspended in lysis buffer containing EDTA (1 mM), and DNAse-free RNAse (Sigma) was then added to a final concentration of 1 µg/ml for 1 h at room temperature. Cyquant dye was added for 5 min at room temperature and DNA concentration measured by fluorescence spectroscopy at 480 nm excitation and 520 nm emission wavelengths using a Tecan Genobios microplate reader. Statistical analysis between control and each group were using Student's t-test.

Example 3:

The therapeutic efficacy of candidate cell lines are tested using the unilateral 6-hydroxydopamine-(6-OHDA)-lesion rat model for PD. In each recipient 50,000-300,000 cells of a differentiated dopaminergic phenotype is stereotactically transplanted into the striatum. The hosts are treated with daily i.p. injections of cyclosporin A (10 mg/kg), a treatment which has previously been shown to efficiently diminish problems of immune rejection of human intracerebral xenografts in rats. The cells are treated with the lipid peroxidation inhibitor Tirilazad mesylate which is already used both in experimental and in clinical trials to enhance graft survival.

The experiment demonstrates that AF expanded stem/progenitor cells survive and are integrated appropriately into the host brain.

Functional assessments: Behaviour

Amphetamine-induced rotational behaviour in rodents with unilateral 6-OHDA lesion is probably the most reliable and sensitive indicator of graft survival and function. Rotation is monitored in rats with unilateral 6-OHDA lesions prior to grafting and every 3 weeks after grafting. In addition, the capacity of the grafts to reverse deficits under non-drug-induced conditions is examined in the so-called "stepping test". This experiment demonstrates that AF expanded stem/progenitors can ameliorate symptoms in a rat model of Parkinson's disease.

Dopamine production and release

To assess the intracellular content of neurotransmitter, striatal tissue levels of dopamine and its major metabolites are monitored in post-mortem samples using HPLC in rats 6-12 weeks after grafting.

To monitor the capacity of the grafted neurons to release synthesised dopamine, assessment of extracellular dopamine is examined using the intracerebral microdialysis technique under basal and drug-evoked (KCl, amphetamine, and apomorphine) conditions, in rats that have exhibited graft-induced behavioural recovery. The experiment show the ability of AF expanded stem/progenitor cells to produce dopamine over long periods.

Example 4

To test whether administration of a pharmaceutical substance comprising AF or a polypeptide comprising the amino acid sequence of Formula I can be used to facilitate learning and memory, two groups of rats are subjected to memory testing. One of the groups is treated with AF. Rats from both groups are tested four weeks after BrdU injection during four consecutive days. The rats are tested in a water maze with a video-tracking system. The time to reach the platform (latency) and the length of the swimming path are monitored. The escape platform is hidden 1 cm below the surface of the water at a fixed position. The water is made opaque by adding dry milk powder to the water. The water temperature is kept constant at 22oC throughout the test. Each animal will be tested in four trials each day. Each trial lasts 45 s. Animals that fail to find the hidden platform within 45 s are designated as having a 45-s latency and are put on the platform and allowed to stay there for 15 s. The swim path and latency in finding the platform during the water maze test is analysed.

The hidden-platform version of the water maze task is used to assess spatial performance during the last four days of the experimental period. Animals from both control and experimental groups are subjected to the test. Both total swim path and escape latencies are analysed. The results are expected to be similar with respect to both parameters; thus, only swim paths are presented. Animals treated with AF are supposed to show to perform significantly better on the spatial learning task. The analysis using one-way repeated measures with ANOVA, are expected to demonstrate a significant effect on swim distance in the AF treated group compared with the control group.

SEQUENCE LISTING

Sequence ID NO:1

Met Val Leu Glu Ser Thr Met Val Cys Val Asp Asn Ser
5 10

Glu Thr Met Arg Asn Gly Asp Phe Leu Pro Thr Arg Leu Gln Ala Gln
15 20 25

Gln Asp Ala Val Asn Ile Val Cys His Ser Lys Thr Arg Ser Asn Pro
30 35 40 45

Glu Asn Asn Val Gly Leu Ile Thr Leu Ala Asn Asp Cys Glu Val Leu
50 55 60

Thr Thr Leu Thr Pro Asp Thr Gly Arg Ile Leu Ser Lys Leu His Thr
65 70 75

Val Gln Pro Lys Gly Lys Ile Thr Phe Cys Thr Gly Ile Arg Val Ala
80 85 90

His Leu Ala Leu Lys His Arg Gln Gly Lys Asn His Lys Met Arg Ile
95 100 105

Ile Ala Phe Val Gly Ser Pro Val Glu Asp Asn Glu Lys Asp Leu Val
110 115 120 125

Lys Leu Ala Lys Arg Leu Lys Lys Glu Lys Val Asn Val Asp Ile Ile
130 135 140

Asn Phe Gly Glu Glu Glu Val Asn Thr Glu Lys Leu Thr Ala Phe Val
145 150 155

Asn Thr Leu Asn Gly Lys Asp Gly Thr Gly Ser His Leu Val Thr Val
160 165 170

Pro Pro Gly Pro Ser Leu Ala Asp Ala Leu Ile Ser Ser Pro Ile Leu

CLAIMS

1. Use of a polypeptide comprising an amino acid sequence of Formula I:

X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent
X2 is H, R or K
X3 is S or L
X4 is T or A
X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent,
or a pharmaceutically acceptable salt thereof,

in the manufacture of a medicament for the treatment and/or prevention of a condition associated with or characterised by a pathological loss and/or gain of cells.

2. Use as claimed in claim 1, wherein Formula I has the sequence of one of:

a) amino acids nos. 35-42 of SEQ ID NO:1
b) amino acids nos. 35-46 of SEQ ID NO:1
c) amino acids nos. 36-51 of SEQ ID NO:1
d) amino acids nos. 36-80 of SEQ ID NO:1
e) amino acids nos. 1-80 of SEQ ID NO:1,
or a pharmaceutically acceptable salt thereof.

3. Use as claimed in claim 1 or claim 2, wherein the condition is characterised by displaying a pathological degeneration of, loss of ability and/or loss of control of regeneration of and/or loss of control of regeneration of a differentiated cell and/or tissue, an embryonic stem cell, an adult stem cell, a progenitor cell and/or a cell derived from a stem cell or progenitor cell.

4. Use as claimed in any one of claims 1 to 3 wherein the condition is associated with or characterised by a pathological loss and/or gain of cells in the peripheral nervous system or central nervous system.

5. Use as claimed in any one of claims 1 to 4, wherein the condition is associated with or characterised by a pathological loss and/or gain of neural stem cells or neural progenitor cells.

6. Use as claimed in any one of claims 1 to 4, wherein the condition is associated with or characterised by a pathological loss and/or gain of oligodendroglial, astroglial, and/or neuronal cells and/or cell populations.

7. Use as claimed in claim 6, wherein the condition is associated with or characterised by a pathological loss and/or gain of non-cholinergic neuronal cells, cholinergic neuronal cells and/or glial cells, and/or cell populations.

8. Use as claimed in any one of claims 1 to 7, wherein the condition is caused by damage to the central nervous system or a defect in the central nervous system.

9. Use as claimed in any one of claims 1 to 7, wherein the condition is caused by a traumatic, malignant, inflammatory, auto-immune or degenerative disorder.

10. Use as claimed in any one of claims 1 to 7, wherein the condition is caused by axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS and/or damage to the spinal cord after disease and/or trauma.

11. Use as claimed in any one of claims 1 to 10, wherein said condition is characterised by memory loss.

12. Use as claimed in any one of claims 1 to 8, wherein the condition is multiple sclerosis, asphyxia, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, Alzheimer's disease, stroke, Meniere's disease or demyelinating disorder.

13. Use of a polypeptide comprising an amino acid sequence of Formula I:

X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent
X2 is H, R or K

X3 is S or L
X4 is T or A
X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent,
or a pharmaceutically acceptable salt thereof,
in the manufacture of a medicament for the treatment and/or prevention of multiple sclerosis, asphyxia, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, Alzheimer's disease, stroke, Meniere's disease or demyelinating disorder.

14. Use as claimed in claim 13, wherein Formula I has the sequence of one of:

a) amino acids nos. 35-42 of SEQ ID NO:1
b) amino acids nos. 35-46 of SEQ ID NO:1
c) amino acids nos. 36-51 of SEQ ID NO:1
d) amino acids nos. 36-80 of SEQ ID NO:1
e) amino acids nos. 1-80 of SEQ ID NO:1,
or a pharmaceutically acceptable salt thereof.

15. Use as claimed in any of one of the previous claims, wherein the medicament is formulated for intravenous infusion, intramuscular injection and/or subcutaneous injection.

16. Use as claimed in any one of the previous claims, wherein the medicament is formulated so that the active substance will pass into the ventricles of a patient's brain when it is administered to said patient.

17. Use as claimed in any one of claims 1 to 15, wherein the medicament is formulated so that the active substance will pass into the cerebrospinal fluid of a patient when it is administered to said patient.

18. A method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny from any germinal layer *in vitro*, characterised by treating the isolated cell with a polypeptide comprising an amino acid sequence of Formula I:

X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent
X2 is H, R or K
X3 is S or L
X4 is T or A
X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent, or a pharmaceutically acceptable salt thereof.

19. A method as claimed in claim 18, wherein Formula I has the sequence of one of:

a) amino acids nos. 35-42 of SEQ ID NO:1
b) amino acids nos. 35-46 of SEQ ID NO:1
c) amino acids nos. 36-51 of SEQ ID NO:1
d) amino acids nos. 36-80 of SEQ ID NO:1
e) amino acids nos. 1-80 of SEQ ID NO:1,
or a pharmaceutically acceptable salt thereof.

20. A method as claimed in claim 18 or claim 19, wherein said isolated cell is selected from the group consisting of chondrocytes, myocardial cells, blood cells, neurons, oligodendrocytes, astroglial cells, progenitor cells, stem cells and/or cells derived from progenitor cells or stem cells.

21. A method of treatment and/or prevention of a condition associated with or characterised by a pathological loss and/or gain of cells, comprising administering to a patient in need thereof an effective amount of a polypeptide comprising an amino acid sequence of Formula I:

X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

wherein
X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent
X2 is H, R or K
X3 is S or L
X4 is T or A
X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent, or a pharmaceutically acceptable salt thereof.

22. A method as claimed in claim 21, wherein Formula I has the sequence of one of:

a) amino acids nos. 35-42 of SEQ ID NO:1
b) amino acids nos. 35-46 of SEQ ID NO:1
c) amino acids nos. 36-51 of SEQ ID NO:1
d) amino acids nos. 36-80 of SEQ ID NO:1
e) amino acids nos. 1-80 of SEQ ID NO:1,
or a pharmaceutically acceptable salt thereof.

23. A method as claimed in claim 21 or 22, wherein the condition is characterised by displaying a pathological degeneration of, loss of ability and/or loss of control of regeneration of and/or loss of control of regeneration of a differentiated cell and/or tissue, an embryonic stem cell, an adult stem cell, a progenitor cell and/or a cell derived from a stem cell or progenitor cell.

24. A method as claimed in any one of claims 21 to 23 wherein the condition is associated with or characterised by a pathological loss and/or gain of cells in the peripheral nervous system or central nervous system.

25. A method as claimed in any one of claims 21 to 24, wherein the condition is associated with or characterised by a pathological loss and/or gain of neural stem cells or neural progenitor cells.

26. A method as claimed in any one of claims 21 to 24, wherein the condition is associated with or characterised by a pathological loss and/or gain of oligodendroglial, astroglial, and/or neuronal cells and/or cell populations.

27. A method as claimed in claim 26, wherein the condition is associated with or characterised by a pathological loss and/or gain of non-cholinergic neuronal cells, cholinergic neuronal cells and/or glial cells, and/or cell populations.

28. A method as claimed in any one of claims 21 to 27, wherein the condition is caused by damage to the central nervous system or a defect in the central nervous system.

29. A method as claimed in any one of claims 21 to 27, wherein the condition is caused by a traumatic, malignant, inflammatory, auto-immune or degenerative disorder.

30. A method as claimed in any one of claims 21 to 27, wherein the condition is caused by axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS and/or damage to the spinal cord after disease and/or trauma.

31. A method as claimed in any one of claims 21 to 30, wherein said condition is characterised by memory loss.

32. A method as claimed in any one of claims 21 to 28, wherein the condition is multiple sclerosis, asphyxia, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, Alzheimer's disease, stroke, Meniere's disease or demyelinating disorder.

33. A method of treatment and/or prevention of multiple sclerosis, asphyxia, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, Alzheimer's disease, stroke, Meniere's disease or demyelinating disorder, comprising administering to a patient in need thereof an effective amount of a polypeptide comprising an amino acid sequence of Formula I:

X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent
X2 is H, R or K
X3 is S or L
X4 is T or A
X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent, or a pharmaceutically acceptable salt thereof.

34. A method as claimed in claim 33, wherein Formula I has the sequence of one of:

a) amino acids nos. 35-42 of SEQ ID NO:1
b) amino acids nos. 35-46 of SEQ ID NO:1

c) amino acids nos. 36-51 of SEQ ID NO:1
d) amino acids nos. 36-80 of SEQ ID NO:1
e) amino acids nos. 1-80 of SEQ ID NO:1,
or a pharmaceutically acceptable salt thereof.

35. A method as claimed in any of one of claims 21 to 34, wherein the medicament is formulated for intravenous infusion, intramuscular injection and/or subcutaneous injection.

36. A method as claimed in any one of claims 21 to 34, wherein the medicament is formulated so that the active substance will pass into the ventricles of a patient's brain when it is administered to said patient.

37. A method as claimed in any one of claims 21 to 34, wherein the medicament is formulated so that the active substance will pass into the cerebrospinal fluid of a patient when it is administered to said patient.

38. A method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny from any germinal layer from a patient, characterised by:

a) administering an effective amount of a polypeptide comprising the amino acid sequence of Formula I as defined in claim 1 or claim 2 to said patient prior to isolating said cell;
b) propagating said isolated cell *in vitro*;
followed by
c) transplanting said propagated cells into the same or another patient in need thereof.

39. A method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny from any germinal layer from a patient, characterised by:

a) isolating said cell and/or stem cell progeny from the patient;
b) administering an effective amount of a polypeptide comprising the amino acid sequence of Formula I as defined in claim 1 or claim 2 to said isolated cell *in vitro* and propagating said cells;

followed by

c) transplanting said propagated cells back into the same or another patient in need thereof.

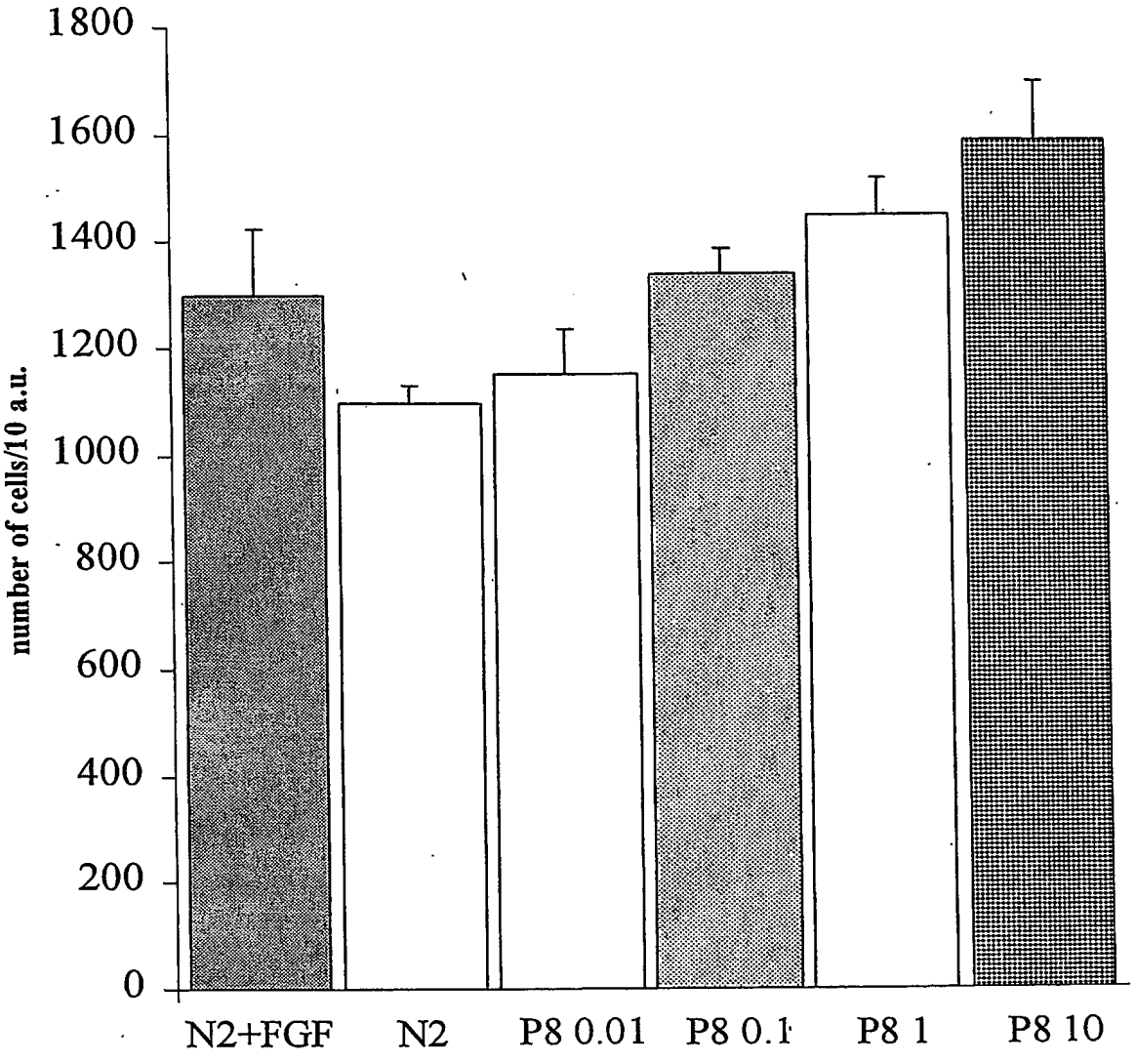
40. A method as claimed in claim 38 or claim 39, wherein said isolated cell is selected from the group consisting of chondrocytes, myocardial cells, blood cells, neurons, oligodendrocytes, astroglial cells, progenitor cells, stem cells and/or cells derived from progenitor cells or stem cells.

ABSTRACT

Use of anti-secretory factor peptides

The invention relates to the use of certain fragments of antisecretory factor (AF) in the manufacture of a medicament for inducing proliferation, apoptosis, differentiation and/or migration of an embryonic stem cell, adult stem cell, progenitor cell and/or a cell derived from a stem cell or progenitor cell, for treating a condition characterised by or associated with loss and/or gain of cells. In a preferred embodiment, the condition is a condition or disease of the CNS and/or PNS, for example, Alzheimer's disease.

Figure 1A


1800
1600
1400
1200
1000
800
600
400
200
0
number of cells/10 a.u.
N2+FGF
N2
P8 0.01
P8 0.1
P8 1
P8 10

5

Figure 1B

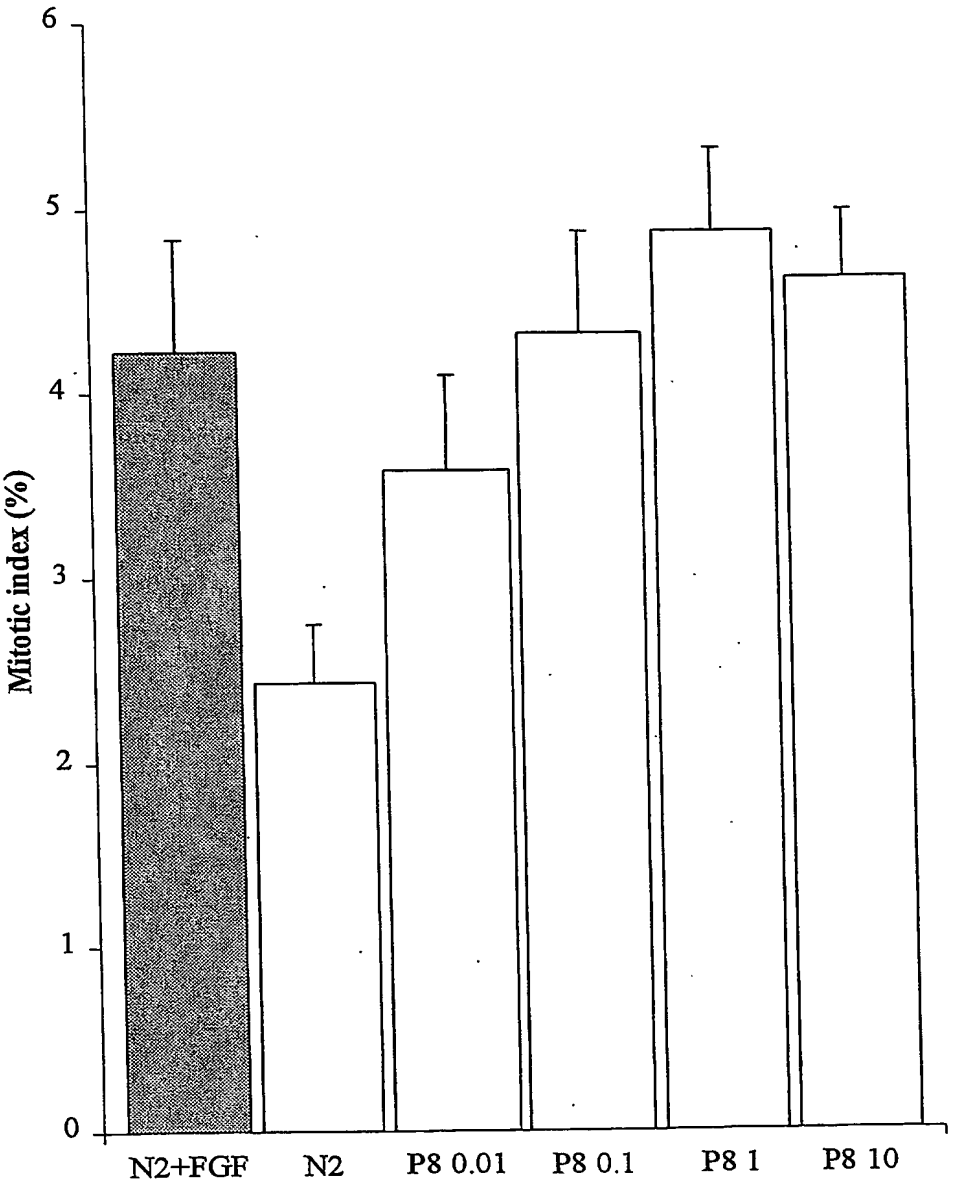


Figure 2A

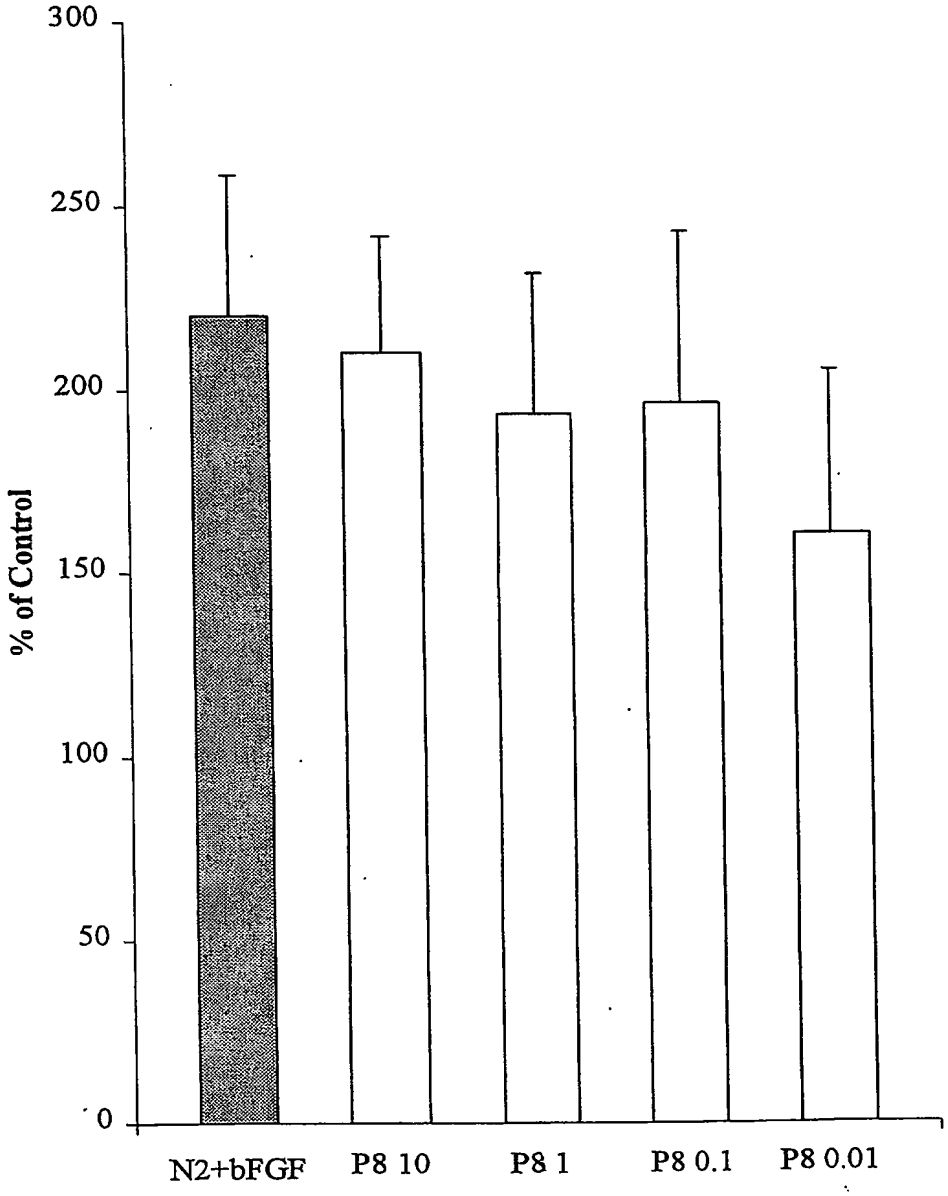
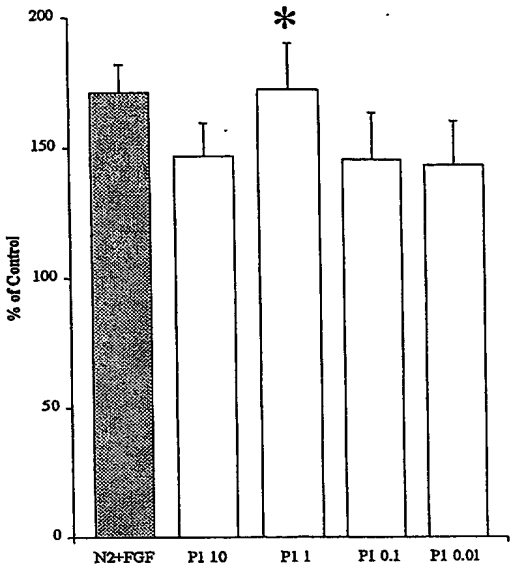


Figure 2B


200
150
100
50
0
% of Control
*
N2+FGF
P1 10
P1 1
P1 0.1
P1 0.01

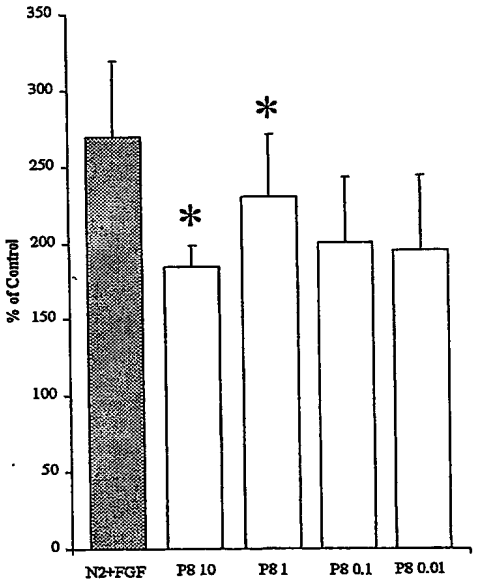

350
300
250
200
150
100
50
0
% of Control
*
*
N2+FGF
P8 10
P8 1
P8 0.1
P8 0.01

Figure 3

```
AATTGGAGGAGT TGTTGTTA GGCCGTCCCG GAGACCCGGT CGGGAGGGAG
GAAGGTGGCAAG ATG GTG TTG GAA AGC ACT ATG GTG TGT GTG GAC AAC AGT  101
             Met Val Leu Glu Ser Thr Met Val Cys Val Asp Asn Ser
                              5                  10
GAG TAT ATG CGG AAT GGA GAC TTC TTA CCC ACC AGG CTG CAG GCC CAG   149
Glu Thr Met Arg Asn Gly Asp Phe Leu Pro Thr Arg Leu Gln Ala Gln
    15                  20                  25
CAG GAT GCT GTC AAC ATA GTT TGT CAT TCA AAG ACC CGC AGC AAC CCT   197
Gln Asp Ala Val Asn Ile Val Cys His Ser Lys Thr Arg Ser Asn Pro
30                  35                  40                  45
GAG AAC AAC GTG GGC CTT ATC ACA CTG GCT AAT GAC TGT GAA GTG CTG   245
Glu Asn Asn Val Gly Leu Ile Thr Leu Ala Asn Asp Cys Glu Val Leu
                50                  55                  60
ACC ACA CTC ACC CCA GAC ACT GGC CGT ATC CTG TCC AAG CTA CAT ACT   293
Thr Thr Leu Thr Pro Asp Thr Gly Arg Ile Leu Ser Lys Leu His Thr
            65                  70                  75
GTC CAA CCC AAG GGC AAG ATC ACC TTC TGC ACG GGC ATC CGC GTG GCC   341
Val Gln Pro Lys Gly Lys Ile Thr Phe Cys Thr Gly Ile Arg Val Ala
        80                  85                  90
CAT CTG GCT CTG AAG CAC CGA CAA GGC AAG AAT CAC AAG ATG CGC ATC   389
His Leu Ala Leu Lys His Arg Gln Gly Lys Asn His Lys Met Arg Ile
    95                  100                 105
ATT GCC TTT GTG GGA AGC CCA GTG GAG GAC AAT GAG AAG GAT CTG GTG   437
Ile Ala Phe Val Gly Ser Pro Val Glu Asp Asn Glu Lys Asp Leu Val
110                 115                 120                 125
AAA CTG GCT AAA CGC CTC AAG AAG GAG AAA GTA AAT GTT GAC ATT ATC   485
Lys Leu Ala Lys Arg Leu Lys Lys Glu Lys Val Asn Val Asp Ile Ile
                130                 135                 140
AAT TTT GGG GAA GAG GAG GTG AAC ACA GAA AAG CTG ACA GCC TTT GTA   533
Asn Phe Gly Glu Glu Glu Val Asn Thr Glu Lys Leu Thr Ala Phe Val
            145                 150                 155
AAC ACG TTG AAT GGC AAA GAT GGA ACC GGT TCT CAT CTG GTG ACA GTG   581
Asn Thr Leu Asn Gly Lys Asp Gly Thr Gly Ser His Leu Val Thr Val
        160                 165                 170
CCT CCT GGG CCC AGT TTG GCT GAT GCT CTC ATC AGT TCT CCG ATT TTG   629
Pro Pro Gly Pro Ser Leu Ala Asp Ala Leu Ile Ser Ser Pro Ile Leu
    175                 180                 185
GCT GGT GAA GGT GGT GCC ATG CTG GGT CTT GGT GCC AGT GAC TTT GAA   677

TTT GGA GTA GAT CCC AGT GCT GAT CCT GAG CTG GCC TTG GCC CTT CGT   725

GTA TCT ATG GAA GAG CAG CGG CAC GCA GGA GGA GGA GCG CGG CGG GCA   733

GCT CGA GCT TCT GCT GCT GAG GCC GGG ATT GCT ACG ACT GGG ACT GAA   821

GAC TCA GAC GAT GCC CTG CTG AAG ATG ACC ATC AGC CAG CAA GAG TTT   869

GGC CGC ACT GGG CTT CCT GAC CTA AGC AGT AGT ACT GAG GAA GAG GAG   917
```

ATT GCT TAT GCC ATG CAG ATG TCC CTG CAG GGA GCA GAG TTT GGC CAG 965

GCG GAA TCA GCA GAC ATT GAT GCC AGC TCA GCT ATG GAC ACA TCT GAG 1013

CCA GCC AAG GAG GAG GAT GAT TAC GAC GTG ATG CAG GAC CCC GAG TTC 1061

CTT CAG AGT GTC CTA GAG AAC CTC CCA GGT GTG GAT CCC AAC AAT GAA 1109

GCC ATT CGA AAT GCT ATG GGC TCC CTG CCT CCC AGG CCA CCA AGG ACG 1157

GCA AGA AGG ACA AGA AGG AGG AAG ACA AGA AGT GAG ACT GGA GGG AAA 1205

GGG TAGC TGAG TCTG CTTA GGGG ACTG GGAA GCAC GGAA TATA GGGT TAGA

TGTG GTTA TCTG TAAC CATT ACAG CCTA AATA AAGC TTGG CAAC TTTT AAAA
AAAA AAAA AAAA AAA